FACTORS INFLUENCING THE RESPONSE OF ISOLATED DOGFISH SKIN MELANOPHORES TO MELANOCYTE-STIMULATING HORMONE[1]

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Although a good deal is known about the endocrinology of melanophores in elasmobranch fishes (Parker, 1948; Waring, 1963), little has been published regarding the hormonal responses of elasmobranch melanophores *in vitro*. The isolated skin of several species of dogfish pales in isotonic media and darkens upon the addition of pituitary extracts containing melanocyte-stimulating hormone (MSH), according to Lundstrom and Bard (1932) and Waring (1936, 1960). This fact correlates well with the established role of MSH in producing the darkening of elasmobranchs upon an illuminated black background. It also shows that dogfish melanophores are similar to frog melanophores in their *in vitro* behavior (Hogben and Winton, 1922). Although the molecular and cellular mechanism of the melanin-dispersing action of MSH is still unknown, MSH may produce melanin dispersion in amphibian melanophores by a sodium-dependent mechanism involving the uptake of water (Novales, 1959, 1962). Osmotic and ionic factors were also found to be of importance for the action of eye stalk hormone on the melanophores of the fiddler crab, *Uca pugnax*, by Fingerman, Miyawaki and Oguro (1963). In view of the relatively primitive nature of elasmobranch fishes, a study has been conducted of osmotic and ionic effects on the response of dogfish melanophores to MSH (Novales and Novales, 1966). It was hoped to learn more about the endocrine cellular physiology of elasmobranch melanophores and possibly to shed light on the evolution of melanophore control mechanisms. Although similarities exist between frog and dogfish melanophores, there are also differences, which could be of theoretical importance.

Materials and Methods

The majority of experiments were performed with the isolated skin from a total of 12 female spiny dogfishes (*Squalus acanthias*). Some work was also done with three smooth dogfishes (*Mustelus canis*). Fishes were caught with hook and line and stored in sea water pens until use. They were kept overnight in an illuminated tank with a light background, resulting in some paling of their skin. The following day the fishes were decapitated. Skin, removed from the dorsolateral trunk region, was placed in elasmobranch Ringer's solution (Cavanaugh, 1956), which had the following composition in g. per l. of distilled water: NaCl,

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16.38; KCl, 0.89; $CaCl_2 \cdot 2H_2O$, 1.47; $NaHCO_3$, 0.38; dextrose, 1.00; urea, 21.6; $NaH_2PO_4 \cdot H_2O$, 0.07. Pieces of skin were mounted on aluminum frames of the type described by Shizume, Lerner and Fitzpatrick (1954) and immersed in 20 ml. of Ringer's solution in a 50-ml. beaker. The inside diameter of the upper elements of these frames had to be about one mm. larger than originally described, due to the greater thickness of dogfish skin. One fish furnished sufficient skin for 24 such frames, enough for a typical experiment of 6 experimental groups. The bath fluid was changed three times in a period of several hours, in order to produce complete blanching of the skin. At the end of this time the experiment was begun, utilizing the paled skin, with highly aggregated melanin in the melanophores. An initial reflectance reading was taken with a Photovolt photoelectric reflection meter, model No. 610, standardized to read 100 with the red filter and porcelain plate provided with the meter. The proper amount of MSH was then added to 20 ml. of Ringer or to the experimental medium. Preliminary time curves showed that full darkening requires 90 minutes. However, since virtually complete darkening had occurred within 60 minutes, the decrease in reflectance in galvanometer units (G.U.) occurring in one hr. (Δ) was used as the standard measure of darkening, as in previous work with frog skin (Novales, 1959). A standard MSH (S-MSH), a highly purified bovine β-MSH (β_b-MSH) and a synthetic α-MSH (syn. α-MSH) were used in the experiments. The S-MSH (Shizume, Lerner and Fitzpatrick, 1954) and β_b-MSH (Geschwind, Li and Barnafi, 1957) were obtained from C. H. Li; the syn. α-MSH (Hofmann and Yajima, 1962) was provided by K. Hofmann. All three hormones were prepared in lactose by the method of Novales, Novales, Zinner and Stoner (1962), to facilitate transporting, weighing and storage. The S-MSH had an activity of 10^9 units/g., when bioassayed by the method of Shizume, Lerner and Fitzpatrick (1954). The other MSH's showed comparable activity after bioassay at the sea shore. In view of the uniformity of the results obtained in the various experiments and with the three MSH's, the results from separate experiments were pooled. However, the majority of experiments were performed with the syn. α-MSH, which had a formyl group on the lysine at position 10.

Microscopic examination of melanophores showed that decrease in reflectance is accompanied by melanin dispersion in the dogfish melanophores. Cleared whole mounts were also prepared in some cases. Sodium analyses of the skin were performed by a similar method to that used for frog skin by Novales, Novales, Zinner and Stoner (1962). Skin was either removed directly from the fish or cut from the experimental frames after treatment. Wet weights were taken and dry weights obtained after drying overnight at 105° C. Samples were digested in one ml. of concentrated nitric acid in a boiling water bath and analyzed for sodium after dilution with distilled water, using a Coleman model 21 flame photometer.

Results

The isolated skin of dogfish darkens when MSH is added to the medium bathing the skin. The log dose-response curves for the three MSH's acting on *Squalus* skin are given in Figure 1. The response to one unit/ml. of syn. α-MSH was significantly greater than the response to the same concentration of S-MSH ($p < 0.05 > 0.01$), but not significantly more than that to β_b-MSH. Since 100

units/ml. produced marked darkening, this concentration was chosen for further experiments on factors influencing the response. Urea was not required for the response. Thus, S-MSH at 10 units/ml. gave a Δ of 28 ± 2 G.U. (2) in urea-free Ringer. The numbers of skins are in parentheses.

The effects of a number of osmotic and ionic variables on the response of *Squalus* skin are given in Table I. There was a very uniform response in Ringer.

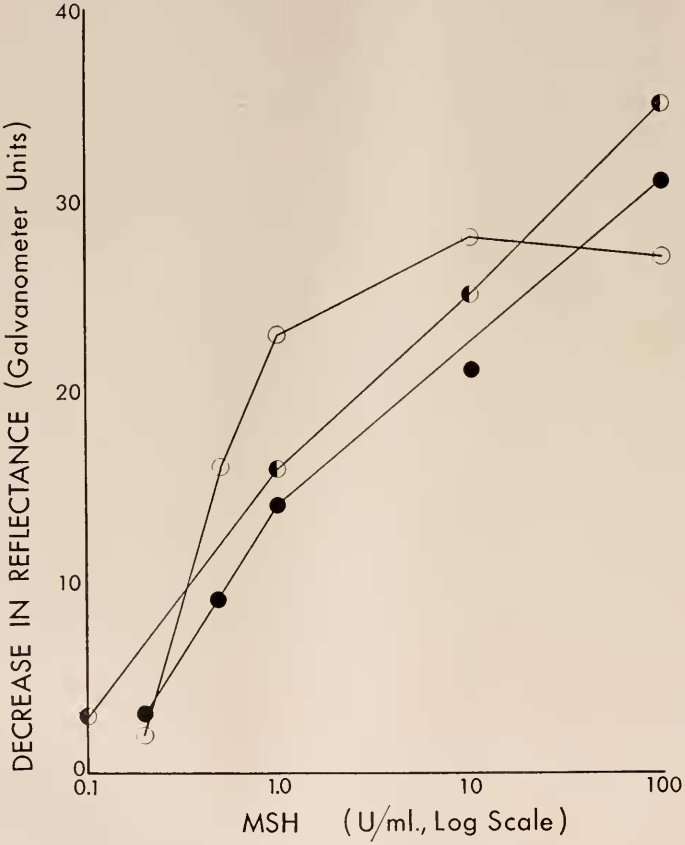

FIGURE 1. Log dose-response curves of paled *Squalus acanthias* skin melanophores to various types of MSH. Exposure was one hour. Points are means. (●) standard MSH, 24 skins; (○) syn. α-MSH, 36 skins; (◐) β_b-MSH, 14 skins.

The sodium content of skin freshly removed from the fish was 753 ± 49 (4) mM/kg. dry wt., which is not significantly different from that found after maintenance in Ringer for hours, as given in Table I. This testifies to the good physiological qualities of the Ringer's solution used here.

Bathing in hypotonic Ringer containing $\frac{1}{4}$ of normal Ringer sodium produced slight darkening, as seen in Table I. MSH produced a normal total darkening in the hypotonic Ringer (Table I). Hypotonic Ringer of $\frac{1}{2}$ and $\frac{3}{4}$ normal Ringer sodium produced no significant darkening alone, showing the resistance of dogfish

melanophores to the darkening effect of hypotonicity. Very dilute Ringer with $\frac{1}{10}$ normal sodium produced a marked darkening of 18 ± 1 (2) G.U. However, MSH failed to produce any more darkening than this, showing the unphysiological nature of this medium. Melanin was dispersed in melanophores treated with the hypotonic Ringer. However, distilled water produced a "cloudy" partially dispersed condition, suggesting that possibly osmotic cytolysis of the melanophores had occurred. The sodium content of skin treated with $\frac{1}{10} \times$ Ringer was 223 ± 16 (5) mM/kg., a marked reduction below that of fresh skin or skin treated with normal Ringer's solution.

Although hypertonicity by itself has no effect on the melanophores, Ringer's solution made hypertonic with sucrose (Table I) blocks the action of MSH. MSH at the lower concentration of 10 units/ml. was also inhibited in the hypertonic

TABLE I

Factors influencing the response of Squalus melanophores to MSH

Medium	1-Hr. darkening (Δ, in G.U.)*		Skin Na after treatment (mM/kg. dry wt.)
	MSH Absent	MSH Present†	
Elasmobranch Ringer	1 ± 0.4 (9)**	29 ± 1 (25)	713 ± 50 (5)
Hypotonic Ringer ($\frac{1}{4} \times$)	8 ± 2 (8)	29 ± 2 (8)	—
Hypertonic Ringer ($2 \times$)	1 ± 1 (7)	11 ± 1 (8)	—
Na-free Ringer (lithium)	1 ± 1 (6)	27 ± 3 (4)	130 ± 10 (4)
Na-free Ringer (choline)	4 ± 1 (8)	26 ± 3 (12)	—
Isotonic†† NaCl (313 mM)	$+1 \pm 1$ (3)	23 ± 3 (4)	—
Isotonic sucrose (625 mM)	6 ± 1 (5)	9 ± 1 (8)	—
Isotonic KCl (313 mM)	8 ± 0.3 (4)	27 ± 2 (4)	146 ± 1 (4)
Isotonic $MgCl_2$ (208 mM)	10 ± 2 (4)	21 ± 2 (4)	146 ± 14 (4)
Isotonic $CaCl_2$ (208 mM)	0 ± 1 (5)	0 ± 0.3 (8)	—

* Decrease in reflectance in galvanometer units.
† MSH concentration of 100 units/ml.
** Figures are means ± standard errors; numbers of skins in parentheses.
†† Isotonic solutions contained urea and dextrose as in Ringer.

medium. Ringer made hypertonic with NaCl to give a final NaCl concentration of 0.56 M completely inhibited MSH action, indicating that a high sodium content is toxic to the melanophores. Furthermore, MSH was markedly inhibited in sea water, which was calculated to be about $1.6 \times$ isotonicity. The hypertonic inhibition was reversible, for skins darkened with MSH upon transfer to normal Ringer after having been in a hypertonic medium.

The effect of replacement of sodium by a variety of other substances is also shown in Table I. Sodium-free Ringer was prepared with LiCl replacing the NaCl, and potassium salts the other sodium salts. MSH was fully active in the lithium Ringer, even though this medium produced an 82% reduction in the sodium content of the skin. MSH was also active in sodium-free choline Ringer (Table I). After these experiments showing that sodium can be replaced in normal Ringer, a simplified type of medium was used, consisting of a single chloride as well as urea and dextrose as in normal Ringer. An isotonic NaCl solution

TABLE II

Factors influencing the response of Mustelus melanophores to MSH

Medium	1-Hr. darkening (Δ, in G.U.)*	
	MSH absent	MSH present†
Elasmobranch Ringer	2 ± 1 (4)**	41 ± 4 (8)
Hypotonic Ringer (¼ ×)	+1 ± 1 (4)	27 ± 3 (5)
Hypertonic Ringer (2 ×)	6 ± 2 (4)	17 ± 2 (7)
Na-free Ringer (choline)	2 ± 2 (4)	32 ± 2 (8)

* Decrease in reflectance in galvanometer units.
† MSH concentration of 100 units/ml.
** Figures are means ± standard errors; numbers of skins in parentheses.

of this type fully supported the response to MSH (Table I). However, there was a two-thirds reduction in the amount of response in an isotonic sucrose medium, showing the need for cations in the response. MSH action was also inhibited in sodium-free Ringer prepared with sucrose, for 10 units/ml. gave a darkening of only 11 ± 2 (4), much lower than the response in normal Ringer, which was about twice as great (Fig. 1). Surprisingly, a strong response was obtained in the isotonic KCl and $MgCl_2$ media (Table I). Either of these solutions alone produced a substantial darkening, probably as a result of their acidity, for their pH values were 6.3 and 6.0, respectively. Shizume, Lerner and Fitzpatrick (1954) found that solutions below a pH of 6.5 will darken frog skin. Both media were effective in reducing the sodium content of dogfish skin. Finally, the $CaCl_2$ medium completely failed to support darkening. This failure was not reversible, indicating that a high calcium concentration is probably toxic to the melanophores.

Similar results were obtained with the skin of the smooth dogfish, *M. canis*, as shown in Table II. *Mustelus* skin gave a greater reflectometric change with MSH than did *Squalus* skin. However, (¼ ×) hypotonic Ringer had no significant darkening alone and the response to MSH was significantly lower in this medium than in normal Ringer ($P = 0.01$). Thus, *Mustelus* is slightly more resistant to

TABLE III

The effect of caffeine on dogfish skin*

Medium	1-Hr. darkening† (Δ, in G.U.)	
	S. acanthias	*M. canis*
Elasmobranch Ringer	19 ± 2 (6)**	16 ± 6 (2)
Na-free Ringer (choline)	26 ± 3 (4)	28 ± 1 (2)
Isotonic†† sucrose (625 m*M*)	15 ± 1 (4)	—
Isotonic $CaCl_2$ (208 m*M*)	0 (4)	—

* Caffeine concentration of 0.01 *M*.
† Decrease in reflectance in galvanometer units.
** Figures are means ± standard errors; numbers of skins in parentheses.
†† Isotonic solutions contained urea and dextrose as in Ringer.

hypotonic darkening than *Squalus*. In hypertonic Ringer there was about a 60% reduction in the response to MSH. However, in sodium-free choline Ringer the response to MSH was not significantly different than in normal Ringer; thus sodium can be replaced in the case of *Mustelus* also.

Experiments with caffeine shed light on the specificity of the above effects on MSH darkening. This agent darkened the skin of both species at 0.01 *M*, as seen in Table III. Moreover, in sodium-free Ringer, there was a greater darkening, although the difference is not statistically significant. This difference was not seen in the isotonic sucrose, however. No response was obtained in the isotonic $CaCl_2$, further demonstrating the toxic effect of this solution on the melanophores.

Discussion

The present study extends earlier results with the isolated skin of *Mustelus canis* and *Scyllium canicula* to *Squalus acanthias*. Lundstrom and Bard (1932) first showed that hypophysectomy causes paling of the dogfish. Melanophores in isolated skin of *M. canis* contracted their pigment in dilute sea water. Melanin dispersion occurred with mammalian or *Mustelus* posterior lobe extract. Waring (1936) obtained similar results with *S. canicula* skin. Because he found that melanin aggregation occurs slowly in dilute sea water, we allowed at least two hours of Ringer rinses to produce maximum paling. Waring (1960) also obtained a graded response to increasing amounts of hormone, as we did in Figure 1. He obtained a good response in Young's elasmobranch saline (dilute sea water and urea) and we did also in our earliest experiments. However, we adopted the elasmobranch Ringer, because of the ease of modifying its composition.

The present results also support the conclusion of Parker (1936) that darkening is brought about in *S. acanthias* by a pituitary hormone and paling by the absence of this hormone, since isolated skin pales maximally when rinsed and darkens maximally upon addition of MSH. Highly purified mammalian hormones act on dogfish melanophores, just as they do on frog melanophores, in confirmation of earlier work showing that a variety of MSH-containing preparations disperse melanin in elasmobranch melanophores (Pickford and Atz, 1957).

The dispensability of urea in the response of *Squalus* melanophores is another indication that the high blood urea of elasmobranchs probably functions solely to maintain osmotic balance (Smith, 1936). Frédéricq (1922) found that the heart of the dogfish *Scyllium catulus* continued to beat in a urea-free salt solution. *Squalus* melanophores are also capable of responding to MSH in the absence of urea.

Waring (1936) observed that dogfish color changes are largely due to changes in the dermal melanophores. The gross and microscopic aspects of color change in *S. acanthias* are illustrated in Waring and Landgrebe (1950). Dogfish color changes require days to occur *in vivo* (Parker, 1948), but only a few hours *in vitro*. Thus, the greater length of time required *in vivo* must be due to the slowness of the control of MSH secretion, rather than to any slowness in the response of melanophores to MSH.

The darkening effect of hypotonic media shows that water entry is capable of producing melanin dispersion in *Squalus* melanophores. Furthermore, *Squalus* melanophores are less sensitive than frog melanophores to the dispersing effect of

hypotonic media. Thus, whereas $\frac{1}{4}\times$ Ringer darkens frog skin about 50% as much as MSH (Novales, 1959), it only darkens *Squalus* skin 28% as much as MSH (Table I). Hypotonic media also disperse melanin in the melanophores of frog (Shizume, Lerner and Fitzpatrick, 1954), bony fish (Spaeth, 1913), fiddler crabs (Fingerman, Miyawaki and Oguro, 1963), and salamanders in tissue culture (Novales and Novales, 1965). This effect has been used to support the view that MSH action may involve the uptake of water by the melanophore (Novales, 1959, 1962). Another way of demonstrating the role of water movement is to show an inhibitory effect of hypertonicity on the response to MSH. This was done in both dogfish species studied (Tables I, II). Hypertonicity inhibits melanin dispersion in frog, fiddler crab and salamander melanophores (*loc. cit.*). However, since dispersion produced by drugs as well as aggregation are inhibited (Novales, 1959), this effect does not establish a role of water entry in MSH action, since the inhibition is not specific to the effect of MSH.

The present study has also shown that dogfish melanophores are able to respond to MSH in sodium-free media. Sodium can be replaced by lithium, choline, potassium or magnesium ions in the response of *Squalus* melanophores, but sucrose or calcium fail to replace sodium (Table I). Thus, a cation must be present for the response to occur. The toxicity of calcium is shown by the failure of caffeine to act in a sodium-free calcium medium, whereas it is effective in a sucrose medium, in contrast to MSH (Table III). The cation requirements for dogfish melanophore responses are thus clearly different from those for frog melanophores. Whereas sodium can be replaced by a variety of other cations in the dogfish response, the requirement for sodium is absolute in the case of the frog response to MSH (Novales, Novales, Zinner and Stoner, 1962). It is unlikely that sufficient sodium was present in the dogfish skin to permit a response to MSH, if sodium were required. About 80% of skin sodium was removed by the sodium-free media (Table I). Since frog skin fails to respond when 90% of its skin sodium is removed (*loc. cit.*), it is unlikely that dogfish skin would respond with 80% of its sodium gone, if sodium were required for the response. Of the ions capable of replacing sodium in the response of *Squalus* skin to MSH, neither lithium, choline or potassium is capable of replacing sodium in the response of frog skin melanophores. The present results also recall those obtained with the fiddler crab by Fingerman, Miyawaki and Oguro (1963). They found that sodium can be replaced by other monovalent cations such as potassium or lithium in the response of melanophores to eyestalk hormone, but divalent cations such as magnesium and calcium fail to replace sodium. Thus, this system differs in that magnesium is able to replace sodium in the dogfish response.

Sodium can be replaced by other cations in a variety of other excitable systems, such as nerve and muscle (Spyropoulos and Tasaki, 1960). However, this does not necessarily mean that sodium ions are not involved in the responses *in vivo*. Thus, sodium has so far been irreplaceable in the response of *Rana pipiens* melanophores to MSH, for virtually all the cations capable of replacing sodium in nerve and muscle excitation have been tried and failed (Novales, 1959; Wright and Lerner, 1960; Novales, Novales, Zinner and Stoner, 1962). On the other hand, the present study has shown that *Squalus* melanophores will respond to MSH when sodium is replaced by a variety of cations. However, these results do not

mean that sodium is not involved in the *Squalus* response *in vivo*. Sodium probably is involved. It merely means that the specificity of the cation-requiring system is broad in *Squalus* but extremely narrow in *Rana*. This difference in specificity could be a reflection of the more primitive nature of the elasmobranch fish when compared with the anuran amphibian. The sodium-requiring process involved in MSH action has apparently increased in its specificity to sodium during evolution. Another possibility is that modern elasmobranchs have lost the high specificity of the sodium requirement possessed by their ancestors. However, this is an unlikely explanation which would be difficult to prove. In view of the phylogenetic position of bony fishes between the cartilaginous fishes and amphibians, information is needed regarding the cation requirements for MSH action in teleost fishes. Information about the requirements in cyclostome fishes would also be of interest, since they are more primitive than elasmobranchs.

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Summary

1. The log dose-response curves to standard MSH, bovine β-MSH, and synthetic α-MSH were obtained for the melanophores of isolated *Squalus acanthias* skin, using a reflectometric technique.

2. Urea is not required for the response of *Squalus* melanophores to MSH, further supporting the view that urea is required solely for maintaining the osmotic balance of elasmobranchs.

3. Hypotonic Ringer ($\frac{1}{10}\times$) produces marked darkening of *Squalus* skin, indicating that water entry can cause melanin dispersion.

4. Hypertonic Ringer ($2\times$) inhibits MSH action on *Squalus* melanophores, indicating that water entry may occur during MSH action.

5. MSH can act on *Squalus* or *Mustelus* melanophores in the absence of sodium and lithium; choline, potassium and magnesium are all capable of replacing sodium in the response of *Squalus* melanophores.

6. MSH action is reduced in a sodium-free sucrose medium; thus there is a cation requirement for MSH action on *Squalus* melanophores.

7. Either there is no sodium requirement for MSH action on the dogfishes studied, or the specificity of the sodium requirement for sodium in the dogfish is much lower than in the frog.

LITERATURE CITED

Cavanaugh, G. M., 1956. Formulae and Methods IV of the Marine Biological Laboratory Chemical Room, Marine Biological Laboratory, Woods Hole, Massachusetts, 61 pp.

Fingerman, M., M. Miyawaki and C. Oguro, 1963. Effects of osmotic pressure and cations on the response of the melanophores in the fiddler crab, *Uca pugnax*, to the melanin-dispersing principle from the sinus gland. *Gen. Comp. Endocr.*, 3: 495–504.

Frédéricq, L., 1922. Pulsations de coeur de *Scyllium catulus* en l'absence d'urée. *Arch. int. Physiol.*, 19: 253–256.

Geschwind, I. I., C. H. Li and L. Barnafi, 1957. The isolation and structure of a melanocyte-stimulating hormone from bovine pituitary glands. *J. Amer. Chem. Soc.*, **79**: 1003–1004.

Hofmann, K., and H. Yajima, 1962. Synthetic pituitary hormones. *Recent Prog. Horm. Res.*, **18**: 41–83.

Hogben, L., and F. R. Winton, 1922. The pigmentary effector system. I-Reaction of frog's melanophores to pituitary extracts. *Proc. Roy. Soc. London, Ser. B*, **93**: 318–329.

Lundstrom, H. M., and P. Bard, 1932. Hypophysial control of cutaneous pigmentation in an elasmobranch fish. *Biol. Bull.*, **62**: 1–9.

Novales, R. R., 1959. The effects of osmotic pressure and sodium concentration on the response of melanophores to intermedin. *Physiol. Zoöl.*, **32**: 15–28.

Novales, R. R., 1962. The role of ionic factors in hormone action on the vertebrate melanophore. *Amer. Zool.*, **2**: 337–352.

Novales, R. R., and B. J. Novales, 1965. The effects of osmotic pressure and calcium deficiency on the response of tissue-cultured melanophores to melanocyte-stimulating hormone. *Gen. Comp. Endocr.*, **5**: 658–676.

Novales, R. R., and B. J. Novales, 1966. Factors influencing the response of dogfish melanophores to MSH. *Amer. Zool.*, **6**: 311–312.

Novales, R. R., B. J. Novales, S. H. Zinner and J. A. Stoner, 1962. The effects of sodium, chloride, and calcium concentration on the response of melanophores to melanocyte-stimulating hormone (MSH). *Gen. Comp. Endocr.*, **2**: 286–295.

Parker, G. H., 1936. Color changes in elasmobranchs. *Proc. Nat. Acad. Sci.*, **22**: 55–60.

Parker, G. H., 1948. Animal Colour Changes and their Neurohumors. Cambridge Univ. Press, Cambridge, Eng., 377 pp.

Pickford, G. E., and J. W. Atz, 1957. The Physiology of the Pituitary Gland of Fishes. N. Y. Zool. Soc., N. Y., 613 pp.

Shizume, K., A. B. Lerner and T. B. Fitzpatrick, 1954. *In vitro* bioassay for the melanocyte-stimulating hormone. *Endocrinology*, **54**: 553–560.

Smith, H. W., 1936. The retention and physiological role of urea in the Elasmobranchii. *Biol. Rev.*, **11**: 49–82.

Spaeth, R. A., 1913. The physiology of the chromatophores of fishes. *J. Exp. Zool.*, **15**: 527–585.

Spyropoulos, C. S., and J. Tasaki, 1960. Nerve excitation and synaptic transmission. *Ann. Rev. Physiol.*, **22**: 407–432.

Waring, H., 1936. Colour changes in the dogfish (*Scyllium canicula*). *Proc. Trans. Liverpool Biol. Soc.*, **49**: 17–64.

Waring, H., 1960. The effect of pituitary extracts on melanophores in isolated elasmobranch skin. *Aust. J. Exp. Biol. Med. Sci.*, **38**: 187–194.

Waring, H., 1963. Color Change Mechanisms of Cold-blooded Vertebrates. Academic Press Inc., N. Y., 266 pp.

Waring, H., and F. W. Landgrebe, 1950. Hormones of the posterior pituitary. *In:* The Hormones, pp. 427–514. Ed. by G. Pincus and K. V. Thimann, Academic Press Inc., N. Y.

Wright, M. R., and A. B. Lerner, 1960. On the movement of pigment granules in frog melanocytes. *Endocrinology*, **66**: 599–609.